

PENNIE & EDMONDS LLP
COUNSELLORS AT LAW
 1155 Avenue of the Americas
 New York, N.Y. 10036-2711
 (212) 790-9090

ATTORNEY DOCKET NO. 8511-021-999Date: April 27, 1999

Assistant Commissioner for Patents
 Box PATENT APPLICATION
 Washington, D.C. 20231

Sir:

The following utility patent application is enclosed for filing:

Applicant(s): Gerald P. Murphy, Alton L. Boynton and Anil Sehgal Executed on: Inventors unavailable at time of execution

Title of Invention: Nr-CAM GENE, NUCLEIC ACIDS AND NUCLEIC ACID PRODUCTS FOR THERAPEUTIC AND DIAGNOSTIC USES FOR TUMORS

PATENT APPLICATION FEE VALUE

TYPE	NO. FILED	LESS	EXTRA	EXTRA RATE	FEE
Total Claims	21	-20	1	\$18.00 each	\$ 18.00
Independent	12	-3	9	\$78.00 each	\$ 702.00
Minimum Fee					
Multiple Dependency Fee If Applicable (\$260.00)					
Total					
50% Reduction for Independent Inventor, Nonprofit Organization or Small Business Concern (a verified statement as to the applicant's status is attached)					
Total Filing Fee					
\$ 0.00					
\$ 1,480.00					
\$ 0.00					
\$ 1,480.00					

U.S.C. § 119. Priority of application nos. 60/083,152 and 60/112,098 filed on 04/27/98 and 12/14/98 is claimed under 35 U.S.C. § 119.

The certified copy of the priority application has been filed in application no. filed

Amend the specification by inserting before the first line the following sentence: This is a continuation-in-part of application no. filed .

Please charge the required fee to Pennie & Edmonds LLP Deposit Account No. 16-1150. A copy of this sheet is enclosed.

Respectfully submitted,

Geraldine F. Baldwin 31,232
 Geraldine F. Baldwin
 (Reg. No.)
 PENNIE & EDMONDS LLP

Enclosure

This form is not for use with continuation, divisional, re-issue, design or plant patent applications.

Please type a plus (+) inside this box -

PTO/SB/29 (12/97)

Approved for use through 09/30/00. OMB 0651-0032

Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

jc504
U.S.
S
66/27/99

UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No. 8511-021-999 Total Pages 165

First Named Inventor or Application Identifier

Murphy, Gerald P.

Express Mail Label No. EM 061 020 777 US

4/27/99

PTO

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

Assistant Commissioner for Patents
ADDRESS TO: Box Patent Application Washington, DC 20231

1. Fee Transmittal Form
Submit an original, and a duplicate for fee processing
2. Specification [Total Pages 136]
 - Descriptive title of the Invention
 - Cross Reference to Related Applications
 - Statement Regarding Fed sponsored R&D
 - Reference to Microfiche Appendix
 - Background of the Invention
 - Brief Summary of the Invention
 - Brief Description of the Drawings (*if filed*)
 - Detailed Description of the Invention (including drawings, *if filed*)
 - Claim(s)
 - Abstract of the Disclosure
3. 26 Drawing(s) (35 USC 113) [Total Sheets 27]
4. Oath or Declaration (unexecuted) [Total Sheets 02]
 - Newly executed (original or copy)
 - Copy from a prior application (37 CFR 1.63(d))
(for continuation/divisional with Box 17 completed)
[Note Box 5 below]
 - i. DELETION OF INVENTOR(S)
Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33 (b).
5. Incorporation By Reference (*useable if Box 4b is checked*)
The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.
17. If a CONTINUING APPLICATION, check appropriate box and supply the requisite information:
 Continuation Divisional Continuation-in-part (CIP) of prior application No: filed.

6. Microfiche Computer Program (*Appendix*)
7. Nucleotide and/or Amino Acid Sequence Submission
(if applicable, all necessary)
 - a. Computer Readable Copy
 - b. Paper Copy (identical to computer copy)
 - c. Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

8. Assignment Papers (cover sheet & document(s))
9. 37 CFR 3.73(b) Statement Power of Attorney
(when there is an assignee)
10. English Translation Document (*if applicable*)
11. Information Disclosure Statement (IDS)/PTO-1449 Copies of IDS Citations
12. Preliminary Amendment
13. Return Receipt Postcard (MPEP 503)
(Should be specifically itemized)
14. Small Entity Statement filed in prior application, Statement(s) Status still proper and desired
15. Certified Copy of Priority Document(s)
(if foreign priority is claimed)
16. Other:

18. CORRESPONDENCE ADDRESS

Customer Number or Bar Code Label

20583

(Insert Customer No. or Attach bar code label here)

or Correspondence address below

NAME			
ADDRESS			
CITY	STATE	ZIP CODE	
TELEPHONE		FAX	

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Box Patent Application, Washington, DC 20231.

Express Mail No.: EM 061 020 777 US

**Nr-CAM GENE, NUCLEIC ACIDS AND NUCLEIC ACID PRODUCTS
FOR THERAPEUTIC AND DIAGNOSTIC USES FOR TUMORS**

5

TABLE OF CONTENTS

	<u>Page</u>
10	
1.	FIELD OF THE INVENTION - 1 -
2.	BACKGROUND OF THE INVENTION - 1 -
15	2.1. BRAIN TUMORS - 1 -
	2.2. CAMs - 4 -
	2.2.1. Nr-CAM - 6 -
20	3. SUMMARY OF THE INVENTION - 9 -
	3.1. DEFINITIONS AND ABBREVIATIONS - 12 -
	4. DESCRIPTION OF THE FIGURES - 13 -
	5. DETAILED DESCRIPTION OF THE INVENTION - 21 -
25	5.1. IDENTIFICATION OF ROLE OF Nr-CAM IN TRANSFORMATION - 24 -
	5.2. THE PRODUCTION OF Nr-CAM NUCLEIC ACIDS, POLYPEPTIDES AND ANTIBODIES AS DIAGNOSTICS, THERAPEUTICS AND COMPONENTS FOR SCREENING ASSAYS - 25 -
30	5.2.1. THE Nr-CAM NUCLEIC ACIDS - 27 -
	5.2.2. EXPRESSION OF Nr-CAM NUCLEIC ACIDS - 36 -

	<u>Page</u>
5.2.3. IDENTIFICATION AND PURIFICATION OF THE Nr-CAM PRODUCTS	- 42 -
5.2.4. ANTIBODIES AND IMMUNE CELLS TO Nr-CAM	- 44 -
5.2.4.1. GENERATION OF ANTIBODIES TO Nr-CAM PROTEINS AND DERIVATIVES THEREOF	- 44 -
5.2.5. Nr-CAM PROTEINS, DERIVATIVES AND ANALOGS	- 48 -
5.3. ASSAYS OF Nr-CAM PROTEINS, DERIVATIVES AND ANALOGS	- 53 -
10 5.4. DIAGNOSIS AND SCREENING	- 54 -
5.5. THERAPEUTIC USES	- 58 -
15 5.5.1. TREATMENT, INHIBITION AND PREVENTION OF DISORDERS INVOLVING OVERPROLIFERATION OF CELLS	- 59 -
5.5.1.1. MALIGNANCIES	- 60 -
5.5.1.2. PREMALIGNANT CONDITIONS	- 63 -
15 5.5.1.3. GENE THERAPY	- 65 -
20 5.5.2. TREATMENT, INHIBITION AND PREVENTION OF HYPERPROLIFERATIVE AND DYSPROLIFERATIVE DISORDERS	- 72 -
20 5.5.2.1. ANTISENSE REGULATION OF Nr-CAM EXPRESSION	- 74 -
25 5.5.2.1.1. Nr-CAM ANTISENSE NUCLEIC ACIDS	- 75 -
25 5.5.2.1.2. THERAPEUTIC USE OF Nr-CAM	- 79 -
5.6. DEMONSTRATION OF THERAPEUTIC OR PROPHYLACTIC UTILITY	- 81 -
30 5.7. THERAPEUTIC/PROPHYLACTIC ADMINISTRATION AND COMPOSITIONS	- 83 -
30 5.7.1. TREATMENT AND PREVENTION OF HYPOPROLIFERATIVE DISORDERS	- 90 -

5.8.	ADDITIONAL USE OF INCREASED Nr-CAM FUNCTION TO PROMOTE INCREASED GROWTH	- 92 -
5.9.	SCREENING FOR Nr-CAM AGONISTS AND ANTAGONISTS	- 93 -
5	5.10. ANIMAL MODELS	- 96 -
6.	EXAMPLE: ISOLATION OF THE Nr-CAM GENE FROM AND CHARACTERIZATION OF ITS EXPRESSION IN HUMAN GLIOBLASTOMA MULTIFORME TUMOR TISSUE	- 98 -
10	6.1. MATERIALS AND METHODS	- 98 -
	6.1.1. HUMAN TISSUES AND CELL LINES	- 98 -
	6.1.2. DIFFERENTIAL DISPLAY POLYMERASE CHAIN REACTION (DD-PCR)	- 98 -
	6.1.3. GENE SPECIFIC RT-PCR	- 99 -
15	6.1.4. NORTHERN BLOT ANALYSIS	- 100 -
	6.1.5. QUANTITATION OF NORTHERN AND SOUTHERN BLOTS	- 101 -
	6.1.6. IN SITU HYBRIDIZATION	- 101 -
	6.1.7. GENOMIC SOUTHERN BLOT	- 102 -
20	6.2. RESULTS	- 103 -
	6.2.1. ISOLATION OF HUMAN Nr-CAM FROM AND DIFFERENTIAL EXPRESSION OF Nr-CAM IN GLIOBLASTOMA	- 103 -
	6.2.2. EXPRESSION OF Nr-CAM IN HUMAN TUMOR TISSUES	- 104 -
25	6.2.3. EXPRESSION OF Nr-CAM IN BRAIN TUMOR CELL LINES	- 104 -
	6.2.4. EXPRESSION OF Nr-CAM IN NORMAL HUMAN BRAIN	- 105 -
30	6.2.5. EXPRESSION OF Nr-CAM IN HUMAN TUMOR CELL LINES	- 105 -

6.2.6.	EXPRESSION OF Nr-CAM IN HUMAN BRAIN TUMORS USING <i>IN SITU</i> HYBRIDIZATION	- 106 -
6.2.7.	GENE AMPLIFICATION	- 108 -
5 7.	EXAMPLE: EFFECT OF REGULATING Nr-CAM EXPRESSION IN GLIOBLASTOMA	- 108 -
7.1.	MATERIALS AND METHODS	- 109 -
7.1.1.	CLONING OF ANTISENSE Nr-CAM	- 109 -
7.1.2.	TRANSFECTION OF GLIOBLASTOMA (GB)	- 110 -
10	7.1.3. CELL MORPHOLOGY	- 110 -
7.1.4.	GROWTH ASSAY	- 110 -
7.1.5.	SOFT AGAR ASSAY	- 111 -
7.1.6.	NORTHERN BLOT ANALYSIS	- 111 -
7.2.	RESULTS	- 112 -
15	7.2.1. EFFECT OF EXPRESSION OF ANTI-SENSE Nr-CAM ON MORPHOLOGY	- 112 -
7.2.2.	EFFECT OF EXPRESSION OF ANTI-SENSE Nr-CAM ON CELL PROLIFERATION	- 114 -
7.2.3.	SOFT AGAR COLONY FORMATION OF Nr-CAM ANTISENSE EXPRESSING CELLS	- 114 -
20	7.2.4. EXPRESSION OF Nr-CAM IN hNr-CAM- ANTISENSE EXPRESSING CELLS	- 115 -
7.2.5.	CELL CYCLE ANALYSIS OF CELLS EXPRESSING hNr-CAM ANTISENSE	- 115 -
25	7.2.6. EFFECT OF ANTISENSE hNR-CAM ON MIGRATION AND INVASION	- 116 -
7.2.7.	EFFECT OF RADIATION OF CELLS ANTISENSE hNr-CAM	- 117 -
30	7.2.8. ANTISENSE hNr-CAM INHIBITS GB TUMOR GROWTH <i>IN VIVO</i>	- 118 -

7.2.9.	INTRATUMORAL INOCULATION OF PLASMID- EXPRESSING ANTISENSE <i>hNr-CAM</i> CAUSED REDUCTION IN GLIOBLASTOMA TUMOR GROWTH	- 119 -
5	7.2.10. ROLE OF <i>hNr-CAM</i> IS NOT CONFINED TO ONE CELL LINE	- 120 -
	7.2.11. ADDITIONAL STUDIES	- 121 -
8.	EXAMPLE: IDENTIFICATION OF GENES ALTERED BY <i>hNr-CAM</i>	- 128 -
10		
15		
20		
25		
30		